IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Li et al.

Examiner: **To Be Assigned**

Group Art Unit: **1614**

Application No.: **10/642,468**

Filed: **August 15, 2003**

Title: **METHOD FOR ASSAYING COMPOUNDS OR AGENTS FOR ABILITY TO DECREASE THE ACTIVITY OF MICROSOMAL PROSTAGLANDIN E SYNTHASE OR HEMATOPOIETIC PROSTAGLANDIN D SYNTHASE**

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450.

Date of Deposit Jan. 21, 2004

Delia Coughlin
(Type or print name of person mailing paper)

Delia Coughlin
(Signature of person mailing paper)

Mail Stop
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

SUBMISSION AND REQUEST FOR ENTRY OF PRIORITY PAPERS 37 C.F.R. § 1.55(a)

Applicants submit herewith a certified copy of **GB application No. 0229244.9**, filed on **December 16, 2002**, for which priority is claimed in the above-identified application.

This submission and request for entry is being made to satisfy the requirements under 35 U.S.C. § 119. Please note that no fees are associated with the entry of the priority documents since they are being timely submitted prior to the date the issue fee is due.

Respectfully submitted,

William C. Coppola, Reg. No. 41,686

Aventis Pharmaceuticals Inc.
Patent Department
Route #202-206 / P.O. Box 6800
Bridgewater, New Jersey 08807-0800
Telephone (908) 231-**4854**
Telefax (908) 231-2626

Aventis Docket No. USAV2002/0098 US NP

INVESTOR IN PEOPLE

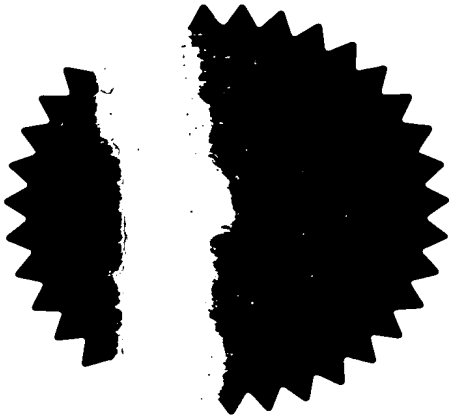
The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed Stephen Hordley.

Dated 1 September 2003

Patents Form 1/77

THE PATENT OFFICE
F
16 DEC 2002
NEWPORT

The Patent Office

160EC02 E771245-1/D10098
P01/7700 0.00-0229244.9

1/77

Patents Act 1977
(Rule 16)

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference — 40/627/P/GB

2. Patent application number *(The Patent Office will fill in this part)* — 0229244.9 — 16 DEC 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

   Aventis Pharmaceuticals Inc
   M.S. 303A
   Route 202-206
   D 303A
   Bridgewater
   New Jersey 08807-0800
   USA

   Patents ADP number *(if you know it)* 79915990004

   If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

   Method for Assaying Compounds for Inhibition of the Activity of Prostaglandin Synthase

5. Name of your agent *(if you have one)*

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

   AdamsonJones

   Broadway Business Centre
   32a Stoney Street
   Nottingham
   NG1 1LL

   Patents ADP number *(if you know it)* 80051180001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
US	US 60/404,808	16 August 2002

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

   a) any applicant named in part 3 is not an inventor, or
   b) there is an inventor who is not named as an applicant, or
   c) any named applicant is a corporate body.
   See note (d))

   Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	20
Claim *(s)*	6
Abstract	1
Drawing *(s)*	8 + 8

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 13 December 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Dr S A Jones 0115 9247 147

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

METHOD FOR ASSAYING COMPOUNDS FOR INHIBITION OF THE ACTIVITY OF PROSTAGLANDIN SYNTHASE

FIELD OF THE INVENTION

The present invention relates to a novel and useful method for assaying compounds and agents for their ability to decrease or inhibit the activity of a prostaglandin synthase.

BACKGROUND OF THE INVENTION

Prostaglandins are a class of eiconisoids that play an important role in pain, fever and inflammation. They are synthesized *in vivo* from arachidonic acid, and possess a five-membered ring of carbon atoms that had formed part of the chain of arachidonic acid. Prostaglandins are not hormones, and act locally, i.e., near the site of their synthesis.

Two particular prostaglandins, PGE_2 and PGD_2, play particularly important roles in the fever, pain and inflammation. In their synthesis *in vivo*, phospholipase A converts a phospholipid into arachidonic acid (found *in vivo* in an ester form). Subsequently, prostaglandin endoperoxide synthase converts arachidonic acid into prostaglandin G_2 (PGG_2). Prostaglandin endoperoxide synthase also catalyzes the reduction of the peroxide group on PGG_2 to form prostaglandin H_2 (PGH_2), which is the precursor of both PGE_2 and PGD_2. In particular, prostaglandin E synthase (PGES) converts PGH_2 into PGE_2 in the presence of cofactor glutathione (GSH), while prostaglandin D synthase (PGDS) converts PGH_2 into PGD_2 in the presence of cofactor GSH.

Since the PGD_2 and PGE_2 play an important role in fever, pain and inflammation, efforts have been made to create assays for compounds that may inhibit their production. In particular, techniques such as HPLC, ELISA or RIA have been used to quantify the production of PGD_2 and PGE_2 in order to determine a compound's or agent's ability to decrease or inhibit the activity of a prostaglandin synthase. However, these techniques possess inherent limitations. For example, they require various washing steps, purification steps and/or usage of radioactive materials. Also, these methods are time-consuming and only have a throughput of tens (HPLC) to several hundred (ELISA & RIA) data points per day. Thus, they are not amenable to high throughput screening.

Fluorescence polarization is a technique that is used to study interactions among molecules. The principles behind this technique are dependent upon the size of molecules being evaluated. In particular, when a fluorescent molecule is illuminated with plane polarized light, electrons at their ground state in the molecule are promoted to an excited state. After approximately 4-5 nanoseconds, these excited electrons decay back to their ground state. It is

during this decay that the molecule emits a fluorescence signal. In fluorescence polarization, this fluorescence emission can be detected only if the molecule remains stationary throughout the excited state. If the molecule moves or rotates during the excited state, the fluorescence emission will be in a different plane of light than that of the polarized light that excited the electrons of the fluorescent aspect. As a result, a fluorescence emission will not be detected. It is well accepted that the smaller the molecule, the greater its mobility and rotation. Hence, a small molecule will produce a substantially smaller signal than a larger molecule, which will remain relatively stationary during the excitation period. It is this property of molecules that fluorescence polarization utilizes. In particular, in a fluorescence polarization assay of a ligand, the ligand, a tracer, i.e., the ligand labeled with a fluorescence label, and the receptor to which the ligand binds, are placed in solution. The ligand and the tracer then compete with each other to bind to the receptor. The solution is then illuminated with plane polarized light, and a signal is then detected. If there is not much ligand present in the solution, the majority of receptors present will bind to the tracer. Since the receptor is a large molecule (relative to the ligand), a signal will be obtained from the fluorescence of the label. In contrast, if there is a large amount of ligand present, then a majority of receptors will bind with the ligand. As a result, a fluorescence signal produced by the tracer, if produced at all, will be substantially smaller than the previously obtained signal produced by the tracer bound to the receptor. It is the difference between these signals that enable one of ordinary skill in the art to determine whether the ligand is present, and its concentration. Fluorescence polarization is measured in millipolarization units, or mP.

Thus, fluorescence polarization can be performed much more simply and efficiently than heretofore known assay methods, such as ELISA, HPLC and RIA. Moreover, it readily lends itself to high throughput screening of a large number of compounds or agents in a very short period of time.

Accordingly what is needed is a fluorescence polarization method for evaluating compounds or agents for their ability to decrease or inhibit the activity of a prostaglandin synthase to produce PGD_2 or PGE_2.

What is also needed is a high throughput system for evaluating the ability of compounds or agents to decrease or inhibit the activity of a prostaglandin synthase. Compounds that inhibit the activity of prostaglandin synthase may readily have applications in treating fever, inflammation, pain, allergy, asthma, rhinitis, sleep disorders, etc.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

SUMMARY OF THE INVENTION

There is provided, in accordance with a present invention, a new and useful method of evaluating compounds or agents for their ability to decrease or inhibit the activity of a prostaglandin synthase to produce a prostaglandin product. Contrary to heretofore known methods, a method of the present invention does not utilize radioactive isotopes, does not require numerous washing steps, can be performed *in vitro*, *in vivo*, in a cell based manner, or in an isolated manner. Moreover, a method of the present invention can readily be performed in a high throughput manner.

Broadly, the present invention extends to a method for determining whether a compound or agent decreases the activity of a prostaglandin synthase to react with its substrate to form a prostaglandin product. Such a method of the present invention comprises the steps of mixing the prostaglandin synthase with its substrate, a cofactor and the compound or agent so that the enzymatic reaction can occur. The mixture is then incubated with a stop solution comprising an agent that prevents the spontaneous conversion of unreacted substrate into the prostaglandin product. This mixture is then incubated with a detection reagent that comprises the prostaglandin product labeled with a fluorescence label (i.e. a tracer), and an antibody having the prostaglandin product as an immunogen. Subsequently, the mixture and a control mixture that has been treated in the identical fashion but lacks the compound or agent, are illuminated with plane polarized light having a wavelength at which the fluorescence label fluoresces. The fluorescence polarization of the mixture and the control mixture are measured and compared. A mixture having a polarization measurement greater than the polarization measurement of the control mixture indicates that the compound or agent decreased the activity of the prostaglandin synthase enzyme. Consequently, such a compound or agent may readily have applications in treating a subject suffering from pain, inflammation, fever, arthritis, asthma, rhinitis, or sleep disorders.

Naturally, the present invention extends to a method such as described above, wherein the prostaglandin synthase is prostaglandin E synthase (PGES), the substrate is prostaglandin H_2 (PGH_2), the cofactor is glutathione (GSH), and the prostaglandin product is prostaglandin E_2 (PGE_2). Alternatively, in a method of the present invention, prostaglandin synthase is

prostaglandin D synthase (PGDS), the substrate is PGH_2, the cofactor is GSH, and the prostaglandin product is prostaglandin D_2 (PGD_2).

As explained above, in a method of the present invention, the stop solution comprises an agent that prevents the spontaneous conversion of unreacted substrate into prostaglandin product. In particular, the substrate for the two prostaglandin synthases described above, PGH_2, contains a peroxide group. A prostaglandin synthase catalyzes the rupture of the oxygen bond of the peroxide group, and the conversion of PGH_2 into either PGD_2 or PGE_2, depending upon which prostaglandin synthase is used. However, PGH_2 also undergoes spontaneous conversion into PGE_2 or PGD_2. This spontaneous conversion can interfere with and alter the results of an assay. Thus, the mixture is incubated with a stop solution comprising an agent that prevents the spontaneous conversion of PGH_2 into either PGD_2 or PGE_2. A particular example of such an agent is $FeCl_2$ at a concentration of about 20 mM. However, one of ordinary skill in the art may readily be familiar with other agents that prevent this simultaneous conversion, and which are encompassed by a method of the present invention. Moreover, the duration of this incubation must be sufficiently long to permit the agent to prevent the conversion of all unreacted substrate into product. In a particular embodiment of the present invention, the incubation has a duration of at least about 30 seconds.

Furthermore, the present invention extends to a method for determining whether a compound or agent decreases the activity of a prostaglandin synthase to react with its substrate to form a prostaglandin product, wherein after incubation with the stop solution, the mixture is incubated with a detection reagent that comprises the prostaglandin product labeled with a fluorescence label, and an antibody having the prostaglandin product as an immunogen. Numerous fluorescence labels known to those of ordinary skill in the art have application s in a method of the present invention. Examples of such fluorescence labels having applications in a method of the present invention include phycoerythrin (PE), Texas red (TR), rhodamine, a free lanthanide series salt, a chelated lanthanide series salt, CyDye (Amersham Biotech), BODIPY (Molecular Probes) and ALEXA (Molecular Probes) to name only a few. In a particular embodiment, the fluorescence label is Texas Red. Moreover, the fluorescence label may be bound directly to the prostaglandin product, or alternatively, bound to a linker molecule, which in turn is bound to the prostaglandin product. Particular linker molecules having applications herein include, but certainly are not limited to aminobutyric acid, aminocaproic acid, 7-aminoheptanoic acid, 8-aminocaprylic acid, Fmoc-aminocaproic acid,

one or more β-alanines, an isothiocyanate group, an isothiocyanate group, a succinimidyl ester, a sulfonal halide or a carbodiimide, to name only a few. In addition, in this step of a method of the present invention, the antibody can be a monoclonal or a polyclonal antibody. This step involves competitive binding between the detectably labeled prostaglandin product and the prostaglandin product produced from the substrate due to catalysis by the prostaglandin synthase, and the duration of this incubation is at least about 30 seconds. However, as described *infra*, this duration may be greater.

Furthermore, the present invention extends to a method for determining whether a compound or agent decreases the activity of a prostaglandin synthase to react with its substrate to form a prostaglandin product. Initially, the method comprises mixing the prostaglandin synthase with the substrate, a cofactor and the compound or agent. This mixture is then incubated with a stop solution that comprises an agent that prevents the spontaneous conversion of unreacted substrate into the prostaglandin product. After the incubation with the stop solution, the mixture is incubated with a detection reagent that comprises the prostaglandin product labeled with Texas Red and an antibody having the prostaglandin product as an immunogen. After this incubation, the mixture and a control mixture are illuminated with plane polarized light at a wavelength of 580 nm, and the fluorescence polarization of the mixture and the control mixture is measured at the emission wavelength of the fluorescence label. In the case of Texas red, it is measured at 620 nm. These fluorescence polarization measurements are compared to determine whether the fluorescence polarization of the mixture is greater than the fluorescence polarization of the control mixture. If so, such a result indicates that the compound or agent decreased the activity of the prostaglandin synthase.

In addition, the present invention extends to a method for determining whether a compound or agent inhibits the reaction of prostaglandin E synthase (PGES) with its prostaglandin H_2 (PGH_2) substrate to form prostaglandin E_2 (PGE_2), comprising the steps of:

(a) mixing PGES with PGH_2, glutathione (GSH) and the compound or agent;

(b) incubating the mixture of step (a) with a stop solution comprising $FeCl_2$ for at least about 30 seconds;

(c) incubating the mixture of step (b) with a detection reagent comprising PGE_2 labeled with Texas Red, and an antibody having PGE_2 as an immunogen for at least about 3 minutes;

(d) illuminating the mixture of step (c) and a control mixture with plane polarized light at a wavelength of 580 nm and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

(e) comparing the measurements of step (d).

The fluorescence polarization measurement of the mixture of step (d) having a value greater than the fluorescence polarization measurement of control mixture indicates the compound or agent decreases the activity of PGES.

In addition, the present invention extends to a method for determining whether a compound or agent inhibits the reaction of prostaglandin D synthase (PGDS) with its prostaglandin H_2 (PGH_2) substrate to form prostaglandin D_2 (PGD_2), comprising the steps of:

(a) mixing PGDS with PGH_2, GSH and the compound or agent;

(b) incubating the mixture of step (a) with a stop solution comprising $FeCl_2$ for at least about 30 seconds;

(c) incubating the mixture of step (b) with a detection reagent comprising PGD_2 labeled with Texas Red, and an antibody having PGD_2 as an immunogen for at least about 1 minute;

(d) illuminating the mixture of step (c) and a control mixture with linearly polarized light at a wavelength of 580 nm and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

(e) comparing the measurements of step (d).

The fluorescence polarization measurement of the mixture of step (d) having a value greater than the fluorescence polarization measurement of control mixture indicates the compound or agent decreases the activity of PGDS.

Furthermore, the present invention extends to a method for determining whether a compound or agent decreases or inhibits the activity of a prostaglandin synthase to produce a prostaglandin product, wherein such a method is performed in a high throughput manner.

Accordingly, it is an aspect to provide a method for evaluating the ability of compounds or agents to decrease or inhibit the activity of a prostaglandin synthase. Consequently, a method of the present invention permits one of ordinary skill in the art to identify a compound or agent that may have applications in treating pain, inflammation, or fever in a subject.

It is another aspect of the present invention to provide a method for evaluating the ability of compounds or agents to decrease or inhibit the activity of a prostaglandin synthase, wherein such a method does not require washing steps or the use of radioactive isotopes.

It is still another aspect of the present invention to provide a method for evaluating the ability of compounds or agents to decrease or inhibit the activity of a prostaglandin synthase that can be performed *in vivo*, *in vitro*, cell based, or in an isolated fashion.

It is yet still another aspect of the present invention to provide a method for evaluating the ability of compounds or agents to decrease or inhibit the activity of a prostaglandin synthase that can be performed in a high throughput manner.

These and other aspects of the present invention will be better appreciated by reference to the following drawings and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematical view of the spontaneous conversion of substrate PGH_2 into either prostaglandin product PGD_2 or PGE_2, when the enzyme utilized is PGES, and the prevention of this spontaneous conversion with $FeCl_2$.

FIG. 2 is a schematical view of a method of the present invention, wherein the prostaglandin synthase is PGES, and the prostaglandin product is PGE_2.

FIG. 3 is a schematical view of the spontaneous conversion of substrate PGH_2 into either prostaglandin product PGD_2 or PGE_2, when the enzyme utilized is PGDS, and the prevention of this spontaneous conversion with $FeCl_2$.

FIG. 4 is a schematical view of a method of the present invention, wherein the prostaglandin synthase is PGDS, and the prostaglandin product is PGD_2.

FIG. 5 shows the chemical structure of MK-886, a known inhibitor of PGES that was used to prove a method of the present invention enables one to determine whether a compound or agent decreases or inhibits the activity a prostaglandin synthase.

FIG. 6 is a graphical view of the concentration response curve of a method of the present invention using the prostaglandin synthase PGES and a known inhibitor MK-886. IC50 = 27.5 uM. These results show that a method of the present invention permits one of ordinary skill in the art to determine whether a compound or agent decreases the activity a prostaglandin synthase, in this case PGES.

FIG. 7 shows the chemical structure of HQL 79.

FIG. 8 shows a histogram showing that the decrease in activity of PGDS caused by HQL 79 can be detected by a method of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based upon the discovery that surprisingly and unexpectedly, fluorescence polarization can be used to identify compounds or agents that decrease the activity of a prostaglandin synthase to produce a prostaglandin, e.g., PGD_2 or PGE_2. Hence broadly, the present invention extends to a method for determining whether a compound or agent decreases the activity of a prostaglandin synthase to react with its substrate to form a prostaglandin product, comprising the steps of:

(a) mixing the prostaglandin synthase with its substrate, a cofactor and the compound or agent;

(b) incubating the mixture of step (a) with a stop solution comprising an agent that prevents the spontaneous conversion of the substrate into the prostaglandin product;

(c) incubating the mixture of step (b) with a detection reagent comprising the prostaglandin product labeled with a fluorescence label and an antibody having the prostaglandin product as an immunogen;

(d) illuminating the mixture of step (c) and a control mixture with linearly polarized light at a wavelength at which the fluorescence label fluoresces, and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

(e) comparing the measurements of step (d),

wherein the fluorescence polarization measurement of the mixture of step (d) that is greater than the fluorescence polarization measurement of control mixture indicates the compound or agent decreases the activity of the prostaglandin synthase.

Numerous terms and phrases used throughout the instant Specification and appended Claims are defined below. Accordingly:

As used herein, the terms "compound" or "agent" refer to any composition presently known or subsequently discovered. Examples of compounds or agents having applications herein include organic compounds (e.g., man made, naturally occurring and optically active), peptides (man made, naturally occurring, and optically active, i.e., either D or L amino acids), carbohydrates, nucleic acid molecules, etc.

As used herein, the term "enzyme" refers to a biomolecule, such as a protein or RNA, that catalyzes a specific chemical reaction. It does not affect the equilibrium of the catalyzed reaction. Rather, the enzyme enhances the rate of reaction by lowering the energy of activation.

As used herein, the term "prostaglandin synthase" refers to an enzyme that catalyzes the conversion of prostaglandin H_2 (PGH_2) into a prostaglandin product. Examples of prostaglandin synthases having applications in the present invention include prostaglandin E synthase (PGES), which converts prostaglandin H_2 into prostaglandin E_2 (PGE_2) in the presence of cofactor glutathione (GSH). Another example is prostaglandin D synthase (PGDS), which converts prostaglandin H_2 into prostaglandin D_2 (PGD_2) in the presence of cofactor GSH.

As used herein, the term "substrate" refers to the compound an enzyme acts upon to produce the product. An example of a substrate having applications herein is PGH_2.

As used herein, the term "cofactor" refers to an inorganic ion or a coenzyme required for enzyme activity. A coenzyme is an organic compound required for enzymatic activity of an enzyme. In a particular embodiment, the cofactor is glutathione (GSH).

As used herein, the term "prostaglandin product" refers to a product produced due action of a prostaglandin synthase and glutathione on the substrate of the prostaglandin synthase.

As used herein, the term "fluorescence label" refers to chemical that fluoresces when illuminated with a particular wavelength of light, wherein the compound is bound directly to a compound of interest, or alternatively, is bound to a linker that is in turn bound to the compound of interest. Examples of fluorescence labels having applications in a method of the present invention include, but certainly are not limited to phycoerythrin (PE), Texas red (TR), rhodamine, a free lanthanide series salt, a chelated lanthanide series salt, BODIPY, ALEXA, CyDye, etc. A particular fluorescence label having applications in a method of the present invention is Texas red.

As used herein, the terms "linker" and "linker molecule" may be used interchangeably, and refer to a chemical moiety to which the fluorescence label and the compound of interest, e.g., the prostaglandin product, are bound. Particular examples of linkers having applications in the present invention include aminobutyric acid, aminocaproic acid, 7-aminoheptanoic acid, 8-aminocaprylic acid, Fmoc-aminocaproic, one or more β-alanines, an isothiocyanate group, a succinimidyl ester, a sulfonal halide, or a carbodiimide, to name only a few. A particular example of a linker having applications in the present invention is a carbodiimide group.

As used herein, the term "control mixture" refers to a mixture containing the same reagents, compounds, cells, etc. in the same amounts as the mixture containing the compound or agent being assayed, and is treated in the same manner as the mixture containing the compound or agent being assayed, except, the control mixture does not contain the compound or agent.

Antibodies

As explained above, a method of the present invention utilizes an antibody having a prostaglandin product, such as PGD_2 or PGE_2 as an immunogen. Such an antibody can be a monoclonal antibody, a polyclonal antibody, or even a chimeric antibody. Various procedures known in the art may be used for the production of polyclonal antibodies to a prostaglandin product such as PGE_2 or PGD_2. For the production of antibody, various host animals can be immunized by injection with the prostaglandin product, including but not limited to rabbits, mice, rats, sheep, goats, etc. In a particular embodiment, the prostaglandin product can be conjugated to an immunogenic carrier, *e.g.*, bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin,

pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, BCG (*bacille Calmette-Guerin*) or *Corynebacterium parvum*.

For preparation of monoclonal antibodies directed toward a prostaglandin product, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. These include, but are not limited to, the hybridoma technique originally developed by Kohler and Milstein [*Nature* **256**:495-497 (1975)], as well as the trioma technique, the human B-cell hybridoma technique [Kozbor et al., *Immunology Today* **4**:72 1983); Cote et al., *Proc. Natl. Acad. Sci. U.S.A.* **80**:2026-2030 (1983)], and the EBV-hybridoma technique to produce human monoclonal antibodies [Cole et al., in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)]. Furthermore, monoclonal antibodies can be produced in germ-free animals utilizing technology described in PCT/US90/02545. Techniques developed for the production of "chimeric antibodies" [Morrison et al., *J. Bacteriol.* **159**:870 (1984); Neuberger et al., *Nature* **312**:604-608 (1984); Takeda et al., *Nature* **314**:452-454 (1985)] by splicing the genes from a mouse antibody molecule specific for a prostaglandin product together with genes from a human antibody molecule of appropriate biological activity can be used.

Conditions

As explained above, a method of the present invention can be performed *in vivo*, *in vitro,* or in an isolated form, wherein all the reagents, enzymes, substrates, etc. were previously isolated and maintained in a buffer solution, such as TRIS, TRIS HCl, HEPEs, or phosphate buffer under physiological conditions (i.e., physiological pH, temperature, etc.), or in a cell-based manner. In a cell based assay, a method of the present invention is used to determine whether the compound or agent being assayed prevents or decreases the cell's secretion of prostaglandin product, while in an *in vitro* method, cells may be lysed prior to performance of a method of the present invention so that a compound or agent can be evaluated in an intracellular medium.

Search of Libraries for Candidate Compounds or Agents that Decrease or Inhibit the Activity of a Prostaglandin Synthase

Conventionally, new chemical entities with useful properties are generated by identifying a chemical compound (called a "lead compound") with some desirable property or activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. However, the current trend is to shorten the time scale for all aspects of drug discovery. Because of the ability to test large numbers quickly and efficiently, high

throughput screening (HTS) methods are replacing conventional lead compound identification methods.

In a particular embodiment, high throughput screening methods involve providing a library containing a large number of potential therapeutic compounds (candidate compounds). Such "combinatorial chemical libraries" are then screened with a method of the present invention to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

Combinatorial chemical libraries

Combinatorial chemical libraries are a preferred means to assist in the generation of new chemical compound leads. A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks called amino acids in every possible way for a given compound length (*i.e.*, the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks. For example, one commentator has observed that the systematic, combinatorial mixing of 100 interchangeable chemical building blocks results in the theoretical synthesis of 100 million tetrameric compounds or 10 billion pentameric compounds (Gallop *et al.* (1994) 37(9): 12331250).

Preparation of combinatorial chemical libraries is well known to those of ordinary skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (*see, e.g.,* U.S. Patent 5,010,175, Furka (1991) *Int. J. Pept. Prot. Res.*, 37: 487-493, Houghton *et al.* (1991) *Nature*, 354: 84-88). Peptide synthesis is by no means the only approach envisioned and intended for use with the present invention. Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (PCT Publication No WO 91/19735, 26 Dec. 1991), encoded peptides (PCT Publication WO 93/20242, 14 Oct. 1993), random biooligomers (PCT Publication WO 92/00091, 9 Jan. 1992), benzodiazepines (U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al.,* (1993) *Proc. Nat. Acad. Sci. USA* 90: 69096913), vinylogous polypeptides (Hagihara *et al.* (1992) *J. Amer. Chem. Soc.* 114:

6568), nonpeptidal peptidomimetics with a Beta D Glucose scaffolding (Hirschmann *et al.*, (1992) *J. Amer. Chem. Soc.* 114: 92179218), analogous organic syntheses of small compound libraries (Chen *et al.* (1994) *J. Amer. Chem.* Soc. 116: 2661), oligocarbamates (Cho, et al., (1993) *Science* 261:1303), and/or peptidyl phosphonates (Campbell *et al.*, (1994) *J. Org. Chem.* 59: 658). *See, generally,* Gordon *et al.*, (1994) *J. Med. Chem.* 37:1385, nucleic acid libraries, peptide nucleic acid libraries (*see, e.g.,* U.S. Patent 5,539,083) antibody libraries (*see, e.g.,* Vaughn *et al.* (1996) *Nature Biotechnology*, 14(3): 309-314), and PCT/US96/10287), carbohydrate libraries (*see, e.g.,* Liang *et al.* (1996) *Science*, 274: 1520-1522, and U.S. Patent 5,593,853), and small organic molecule libraries (*see, e.g.,* benzodiazepines, Baum (1993) C&EN, Jan 18, page 33, isoprenoids U.S. Patent 5,569,588, thiazolidinones and metathiazanones U.S. Patent 5,549,974, pyrrolidines U.S. Patents 5,525,735 and 5,519,134, morpholino compounds U.S. Patent 5,506,337, benzodiazepines 5,288,514, and the like).

Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.,* 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). A number of well known robotic systems have also been developed for solution phase chemistries. These systems include automated workstations like the automated synthesis apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, Mass.; Orca, HewlettPackard, Palo Alto, Calif.) which mimic the manual synthetic operations performed by a chemist. Any of the above devices are suitable for use with the present invention. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art. In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.,* ComGenex, Princeton, N.J., Asinex, Moscow, Ru, Tripos, Inc., St. Louis, MO, ChemStar, Ltd, Moscow, RU, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, *etc.*).

<u>High throughput assays of chemical libraries</u>

Naturally, a method of the present invention, which employs fluorescence polarization, is readily amenable to high throughput screening. High throughput screening systems are commercially available (*see, e.g.,* Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, *etc.*). These systems typically automate entire procedures including all sample and reagent

pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. The manufacturers of such systems provide detailed protocols for the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like.

The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following Examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE I

Fluorescence Polarization Assay of Prostaglandin E Synthase (PGES)

Prostaglandin E_2 (PGE_2) is a major mediator involved in inflammation and pain. PGES catalyzes the conversion of PGH_2 to PGE_2. Expression of microsomal PGE_2 synthase (mPGES) is induced in many inflammatory conditions. Inhibitors to mPGES will provide potential effective therapies for inflammation, pain, fever, and both osteoarthritis and rheumatoid arthritis.

An assay has been developed to measure the conversion of PGH_2 to PGE_2 by PGE_2 synthase. The assay is configured based on the Fluorescence Polarization principle. The enzyme is incubated with PGH_2, glutathione, and the compound or agent being evaluated. After a short incubation period (at least about 30 seconds), a stop solution comprising $FeCl_2$ and citric acid is added to quench any remaining PGH_2, which would otherwise undergo spontaneous conversion to PGD_2 or PGE_2, and thus interfere with the quantification of the enzymatic conversion of PGH_2 to PGE_2 (Figure 1). A detection solution containing a fluorescence labeled (Texas Red) tracer (PGE_2) and anti-PGE_2 antibody is then be added in order to generate the specific signal that is inversely proportional to the production of PGE_2 (Figure 2). The PGE_2 generated from the enzymatic reaction will compete specifically for the antibody and release the fluorescence labeled tracer. Inhibition of PGE_2 synthase activity will result in increased FP value.

Materials

GSH:	available from Sigma (Catalog # G-6529)
PGH_2:	available from Cayman Chemicals, Inc. (Catalog #17020)

PGE_2 synthase: cloned and produced in house

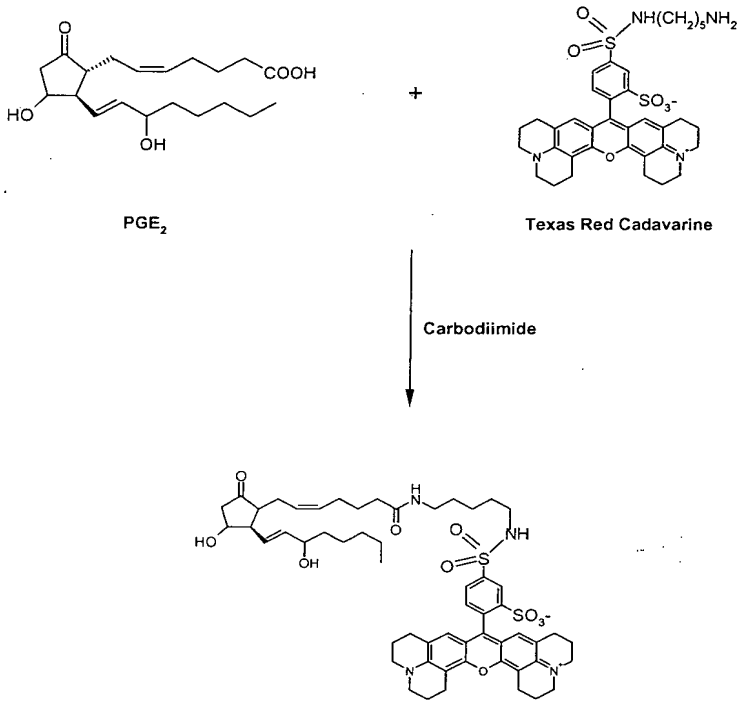
PGE_2 monoclonal antibody: avialable from Assay Designs, Inc.(Catalog # 915-057)

PGE_2: available from Cayman Chemicals, Inc. (Catalog #14010)

Fluorescence labeled Prostaglandin product PGE_2

As explained above, numerous fluorescence labels have applications in a method of the present invention. In a particular embodiment, the fluorescence label Texas Red is linked via a linker to prostaglandin E_2 (PGE_2). In this embodiment, the PGE_2 labeled with Texas Red was synthesized by Combinix (San Mateo, CA).The mechanism for producing such a moiety is described below:

PGES Tracer Synthesis



In this synthesis, Texas Red Cadavarine (Molecular Probes) was added to a solution of the prostaglandin E2 (Cayman Chemicals) in dry methylene chloride. Dicyclohexylcarbodiimide (Sigma-Aldrich) was added and the reaction was stirred under nitrogen in the dark for 24 hours. Purification was performed by reverse phase HPLC chromatography using a water/acetonitrile gradient with 0.05% TFA as a modifier. Naturally, the linker used in this synthesis can be varied.

Method

Initially, the enzyme was diluted in reaction buffer containing K_2HPO_4 and KH_2PO_4 to make a phosphate buffered enzyme solution. The compound or agent being assayed was then placed in the enzyme solution. Optionally, this solution can then be incubated. In a particular example, the incubation period of this solution is about 30 minutes. The substrate PGH_2 in acetone and cofactor GSH were then placed in a separate container at 4° C. The enzyme solution was then added to the container containing PGH_2 in order to start the reaction. This

mixture was incubated for about 30 seconds. Stop solution containing $FeCl_2$ at 20 mM was then mixed into the mixture to prevent spontaneous conversion of any remaining PGH_2 into PGE_2. Detection Solution was then added to the mixture, and the entire mixture was incubated for about 3 minutes. In a particular embodiment, the duration of this incubation may be at least about 120 minutes. A control mixture that is identical to this mixture, except that the control mixture is lacking the compound or agent, was treated identically, i.e., contain the same reagents, had been incubated for the same periods of time, etc. The entire mixture and the control mixture were then illuminated with plane polarized light at a wavelength of 580 nm and the fluorescence polarization of the mixture and the control mixture were measured using a fluorescence filter set with an excitation wavelength of 580 nm, and an emission wavelength of 620 nm. The measurements were made while the measuring instrument was in FP mode. These two measurements were then compared to determine whether the fluorescence polarization measurement of the mixture containing the compound or agent is greater than the fluorescence measurement of the control solution. A measurement of the mixture that is greater than a measurement of the control mixture indicates the compound or agent decreases the activity of PGES.

Results

The assay described above was tested with a known inhibitor of PGES. This inhibitor, MK-886 is commercially available from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA, catalog #EI-266), and has been assigned CAS# 118414-82-7. Previously, MK-886 was determined to be a potent and selective inhibitor of 5-lipoxygenase activating protein. Its structure is set forth in FIG. 5. The results of this experiment are set forth in FIG. 6. These results show the concentration response curve of the PGES assay, and clearly indicate that a method can readily be used to assay a compound or agent's ability to decrease or inhibit the activity of PGES.

EXAMPLE II

Fluorescence Polarization Assay of Prostaglandin D Synthase (PGDS)

Antigenic challenge will increase the production of PGD_2 in airway allergic disorders. PGD_2, which is produced due to prostaglandin D synthase's (PGDS) conversion of PGH_2 into PGD_2, binds to both D type prostaglandin receptor (DP) and the chemokine receptor for Th2 cells (CRTH2), and increases bronchoconstriction, vasodilation, and nasal mucosal dilation. The resulting bronchial hyperactivity, nasal blockage, and eosinophil and Th2 cell infiltration lead to allergic responses. Consequently, compounds or agents that decrease or inhibit the activity of PGDS may readily have applications as therapeutics.

A fluorescence polarization (FP) assay to measure PGDS activity has also been developed (Figure 3 and 4). The assay is configured based on the fluorescence polarization principle. PGDS was mixed with PGH_2, glutathione, and the compound or agent being evaluated. After a short period of time (about 30 seconds), a stop solution comprising $FeCl_2$ and citric acid was added to quench any remaining PGH_2, which would undergo spontaneous conversion to a mixture of PGD_2 and PGE_2, and thus interfere with the quantification of the enzymatic conversion of PGH_2 to PGD_2 (Figure 3). A detection solution containing a fluorescence labeled (Texas Red) tracer (PGD_2) and anti-PGD_2 antibody was then be added in order to generate the specific signal that is inversely proportional to the production of PGD_2 (Figure 4). The PGD_2 generated from the enzymatic reaction competed specifically for the antibody and released the fluorescence labeled tracer. For reasons discussed above, inhibition of PGD_2 synthase activity results in increased fluorescence polarization (FP) value.

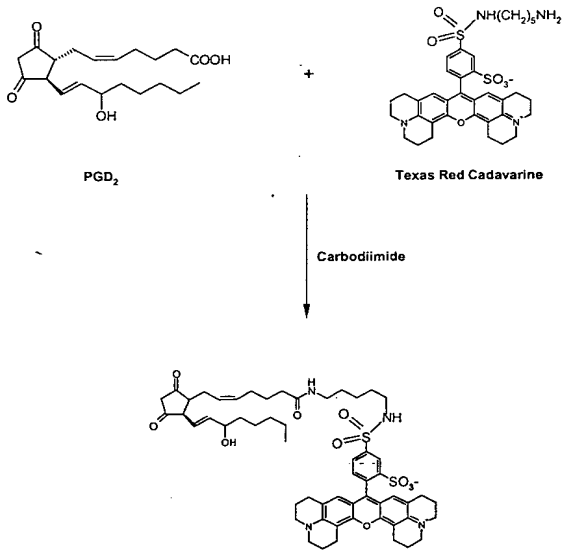
Materials

GSH: available from Sigma (Catalog # G-6529)
PGH_2: available from Cayman Chemicals, Inc. (Catalog #17020)
PGD_2 synthase: cloned and produced in house
PGD_2 monoclonal antibody: available from Institut Pasteur, France
PGD_2: available from Cayman Chemicals, Inc. (Catalog #12010)

Fluorescence labeled Prostaglandin product PGD_2

Just as in Example 1 above, Texas Red was used as the fluorescence label. The prostaglandin D_2 (PGD_2) labeled with Texas red was also produced by Combinix (San Mateo, CA). The mechanism for producing such a moiety is described below:

PGDS Tracer Synthesis



* Link method can be varied

In this synthesis, a Texas Red Cadavarine (Molecular Probes) was added to a solution of the prostaglandin D2 (Cayman Chemicals) in dry methylene chloride. Dicyclohexylcarbodiimide (Sigma-Aldrich) was added and the reaction was stirred under nitrogen in the dark for 24 hours. Purification was performed by reverse phase HPLC chromatography using a water/acetonitrile gradient with 0.05% TFA as a modifier. Naturally, the linker used can be varied.

Method

This method is the same as that used in Example I, except that the enzyme utilized is PGDS, and the prostaglandin product is PGD_2. Thus, as explained above, the enzyme and GSH were initially diluted together in reaction buffer containing K_2HPO4 and KH_2PO4 to make an enzyme solution. The compound or agent being assayed was then placed in this phosphate buffered enzyme solution. Optionally, this solution can then be incubated. The duration of this incubation can vary from a few minutes up to over an hour. In this particular example, the incubation period of this solution was about 30 minutes.

The substrate PGH_2 in acetone was placed in a separate container at 4° C. The enzyme solution was then added to the container containing PGH_2 in order to start the reaction. This mixture was incubated for about 30 seconds. Stop solution comprising $FeCl_2$ and citric acid was then mixed into the mixture to prevent spontaneous conversion of any remaining PGH_2 into PGE_2 or PGD_2. Detection Solution comprising an antibody having PGD_2 as an immunogen and Texas Red labeled PGD_2 (the tracer), was then added to the mixture, and the entire mixture was incubated for at least about 1 minute. However, this incubation step may be up to at least about 120 minutes. Just as in Example 1, a control mixture was prepared that is identical to the mixture containing the enzyme, wherein the control mixture was treated in a manner identical the mixture containing the enzyme, i.e., same incubation durations, etc. However, the control mixture did not contain the compound or agent being evaluated. The entire mixture and the control mixture were then illuminated with plane polarized light at a wavelength of 580 nm (the wavelength at which Texas Red fluoresces), and the fluorescence polarization of the mixture and the control mixture were measured using a fluorescence filter set with an excitation wavelength of 580 nm, and an emission wavelength of 620 nm. The measurement is made while the measuring instrument is in FP mode. Naturally, the wavelengths used will be dependent upon the fluorescence label used in the method. These

two FP measurements are then compared to determine whether the fluorescence polarization measurement of the mixture containing the compound or agent is greater than the fluorescence measurement of the control solution. A measurement of the mixture being greater than a measurement of the control mixture indicates the compound or agent decreases the activity of PGDS.

Results

The assay described above was performed using HQL 79, a known inhibitor of PGDS. HQL 79 is described in WO 95/01350, which is hereby incorporated by reference in its entirety. The structure of HQL 79 is set forth in FIG. 7. The results of this experiment are set forth in FIG. 8. These results clearly show that a method of the present invention detected that HQL 79 decreased the activity of PGDS.

Conclusion

Examples I and II readily demonstrate that a method of the present invention is easy, quick, does not require multiple washings or radioactive isotopes, and can readily be used in a high throughput manner, to determine whether a compound or agent decreases or inhibits the activity of a prostaglandin synthase.

The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A method for determining whether a compound or agent decreases the activity of a prostaglandin synthase to react with its substrate to form a prostaglandin product, comprising the steps of:

(a) mixing the prostaglandin synthase with the substrate, a cofactor and the compound or agent;

(c) incubating the mixture of step (a) with a stop solution comprising an agent that prevents the spontaneous conversion of the substrate into the prostaglandin product;

(c) incubating the mixture of step (b) with a detection reagent comprising the prostaglandin product labeled with a fluorescence label, and an antibody having the prostaglandin product as an immunogen;

(d) illuminating the mixture of step (c) and a control mixture with plane polarized light having a wavelength at which the fluorescence label fluoresces, and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

(f) comparing the measurements of step (d),

wherein the fluorescence polarization measurement of the mixture of step (d) that is greater than the fluorescence polarization measurement of control mixture indicates the compound or agent decreases the activity of the prostaglandin synthase.

2. The method of Claim 1, wherein the prostaglandin synthase is prostaglandin E synthase (PGES), the substrate is prostaglandin H_2 (PGH_2), the cofactor is glutathione, and the prostaglandin product is prostaglandin E_2 (PGE_2).

3. The method of Claim 1, wherein the prostaglandin synthase is prostaglandin D synthase (PGDS), the substrate is PGH_2, the cofactor is GSH, and the prostaglandin product is prostaglandin D_2 (PGD_2).

4. The method of Claim 1, wherein the agent of the stop solution is $FeCl_2$.

5. The method of Claim 1, wherein the incubating step (b) has a duration of at least about 30 seconds.

6. The method of Claim 1, wherein the incubating step (d) has a duration of at least about 30 seconds.

7. The method of Claim 1, wherein the fluorescence label comprises phycoerythrin (PE), Texas red (TR), rhodamine, a free lanthanide series salt, a chelated lanthanide series salt, BODIPY, ALEXA, or CyDye.

8. The method of Claim 1, wherein the fluorescence label is Texas red (TR).

9. The method of Claim 2, wherein the agent of the stop solution is $FeCl_2$.

10. The method of Claim 9, wherein incubating step (b) has a duration of at least about 30 seconds, and the incubating step (c) has a duration of at least about 3 minutes.

11. The method of Claim 10, wherein the fluorescence label comprises phycoerythrin (PE), Texas red (TR), rhodamine, a free lanthanide series salt, a chelated lanthanide series salt, BODIPY, ALEXA, or CyDye.

12. The method of Claim 10, wherein the fluorescence label is Texas red (TR), and the wavelength of the plane polarized light is 580 nm.

13. The method of Claim 3, wherein the agent of the stop solution is $FeCl_2$.

14. The method of Claim 13, wherein incubating step (b) has a duration of at least about 30 seconds, and the incubating step (c) has a duration of at least about 1 minute.

15. The method of Claim 14, wherein the fluorescence label comprises phycoerythrin (PE), Texas red (TR), rhodamine, a free lanthanide series salt, a chelated lanthanide series salt, BODIPY, ALEXA or CyDye.

16. The method of Claim 14, wherein the fluorescence label is Texas red (TR), and the wavelength of the plane polarized light is 580 nm.

17. The method of Claim 2, wherein:

(i) the agent of the stop solution is $FeCl_2$;

(ii) the incubating step (b) has a duration of at least about 30 seconds and the incubating step (c) has a duration of at least about 3 minutes;

(iii) the fluorescence label is Texas Red; and

(iv) the plane polarized light of the illuminating step has a wavelength of 580 nm.

18. The method of Claim 3, wherein:

(i) the agent of the stop solution is $FeCl_2$;

(ii) the incubating step (b) has a duration of at least about 30 seconds and the incubating step (c) has a duration of at least about 1 minute;

(iii) the fluorescence label is Texas Red;

(iv) and the plane polarized light of the illuminating step has a wavelength of 580 nm.

19. A method for determining whether a compound or agent decreases the activity of a prostaglandin synthase to react with its substrate to form a prostaglandin product, comprising the steps of:

(a) mixing the prostaglandin synthase with the substrate, a cofactor and the compound or agent;

(b) incubating the mixture of step (a) with a stop solution comprising an agent that the prevents spontaneous conversion of unreacted substrate into prostaglandin product;

(c) incubating the mixture of step (b) with a detection reagent comprising the prostaglandin product labeled with Texas Red, and an antibody having the prostaglandin product as an immunogen;

(d) illuminating the mixture of step (c) and a control mixture with plane polarized light at a wavelength of 580 nm and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

(e) comparing the measurements of step (d),

wherein the fluorescence polarization measurement of the mixture of step (d) that is greater than the fluorescence polarization measurement of control mixture indicates the compound or agent decreases the activity of the prostaglandin synthase.

20. The method of Claim 19, wherein the prostaglandin product labeled with Texas Red comprises a linker molecule to which the prostaglandin product and the Texas Red are bound.

21. The method of Claim 20, wherein the linker molecule is selected from the group consisting of aminobutyric acid, aminocaproic acid, 7-aminoheptanoic acid, 8-aminocaprylic acid, Fmoc-aminocaproic acid, one or more β-alanines, an isothiocyanate group, an isothiocyanate group, a succinimidyl ester, a sulfonal halide, and a carbodiimide.

22. The method of Claim 19, wherein the prostaglandin synthase is prostaglandin E synthase (PGES), its substrate is prostaglandin H_2 (PGH_2), the cofactor is glutathione and the prostaglandin is prostaglandin E_2 (PGE_2).

23. The method of Claim 22, wherein the incubating step (b) has a duration of at least about 30 seconds and the incubating step (c) has a duration of at least about 3 minutes.

24. The method of Claim 19, wherein the prostaglandin synthase is prostaglandin D synthase (PGDS), the substrate is PGH_2, the cofactor is glutathione and the prostaglandin product is prostaglandin D_2 (PGD_2).

25. The method of Claim 24, wherein the incubating step (b) has a duration of at least about 30 seconds and the incubating step (c) has a duration of at least about 1 minute.

26. The method of Claim 25, wherein the agent of the stop solution is $FeCl_2$.

27. A method for determining whether a compound or agent decreases the activity of prostaglandin E synthase (PGES) to react with its prostaglandin H_2 (PGH_2) substrate to form prostaglandin E_2 (PGE_2), comprising the steps of:

(a) mixing PGES with PGH_2, glutathione, and the compound or agent;

(b) incubating the mixture of step (a) with a stop solution comprising $FeCl_2$ for at least about 30 seconds;

(c) incubating the mixture of step (b) with a detection reagent comprising PGE_2 labeled with Texas Red, and an antibody having PGE_2 as an immunogen for at least about 3 minutes;

(d) illuminating the mixture of step (c) and a control mixture with plane polarized light at a wavelength of 580 nm and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

(e) comparing the measurements of step (d),

wherein he fluorescence polarization measurement of the mixture of step (d) that is greater than the fluorescence polarization measurement of control mixture indicates the compound or agent decreases the activity of PGES.

28. A method for determining whether a compound or agent decreases the activity of prostaglandin D synthase (PGDS) to react with its prostaglandin H_2 (PGH_2) substrate to form prostaglandin D_2 (PGD_2), comprising the steps of:

(a) mixing PGDS with PGH_2, glutathione and the compound or agent;

(b) incubating the mixture of step (a) with a stop solution comprising $FeCl_2$ for at least about 30 seconds;

(c) incubating the mixture of step (b) with a detection reagent comprising PGD_2 labeled with Texas Red, and an antibody having PGD_2 as an immunogen for at least about 1 minute;

(d) illuminating the mixture of step (c) and a control mixture with plane polarized light at a wavelength of 580 nm and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

(e) comparing the measurements of step (d),

wherein the fluorescence polarization measurement of the mixture of step (d) that is greater than the fluorescence polarization measurement of control mixture indicates the compound or agent decreases the activity of PGDS.

ABSTRACT OF THE DISCLOSURE

Provided herein is a novel and useful method for evaluating the ability of compounds or agents to decrease or inhibit the activity of a prostaglandin synthase to produce a prostaglandin product.

FIGURE 1

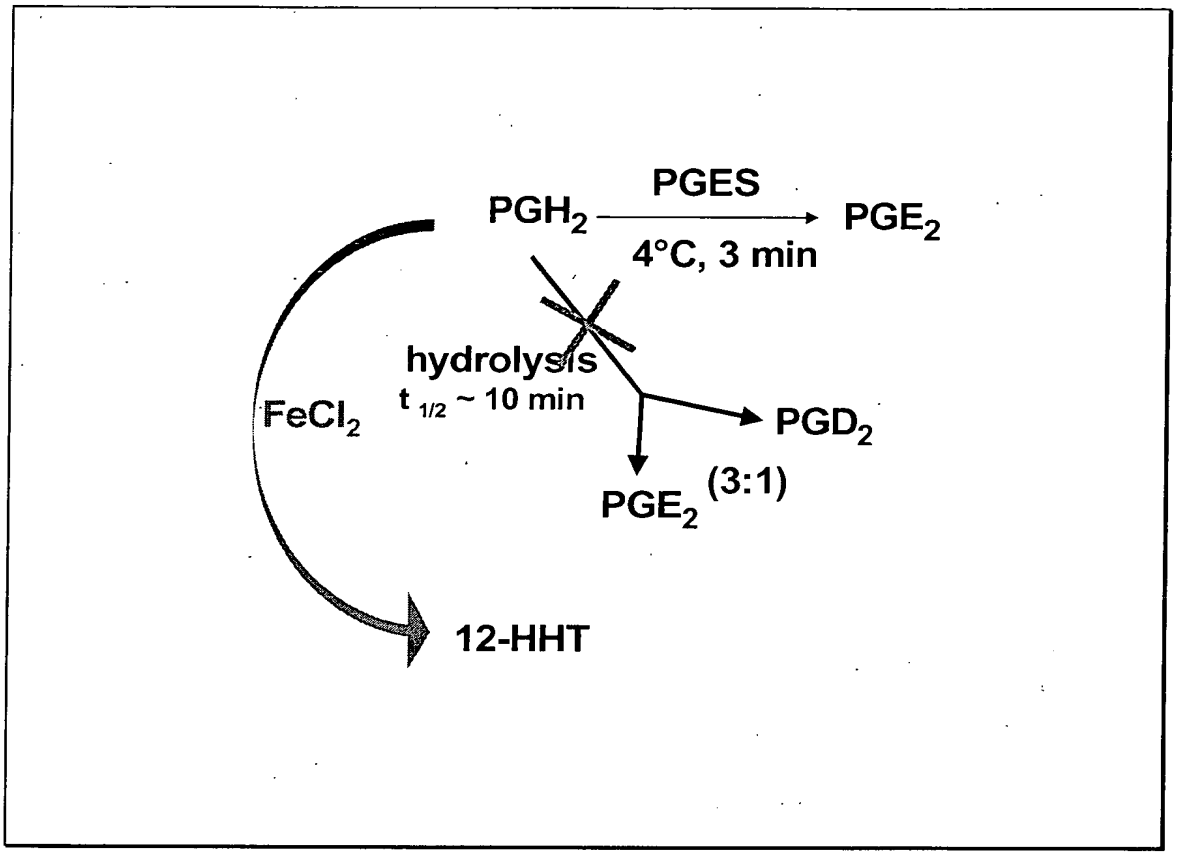

PGES
PGH2
PGE2
4°C, 3 min
hydrolysis
t 1/2 ~ 10 min
FeCl2
PGD2
PGE2 (3:1)
12-HHT

FIGURE 2

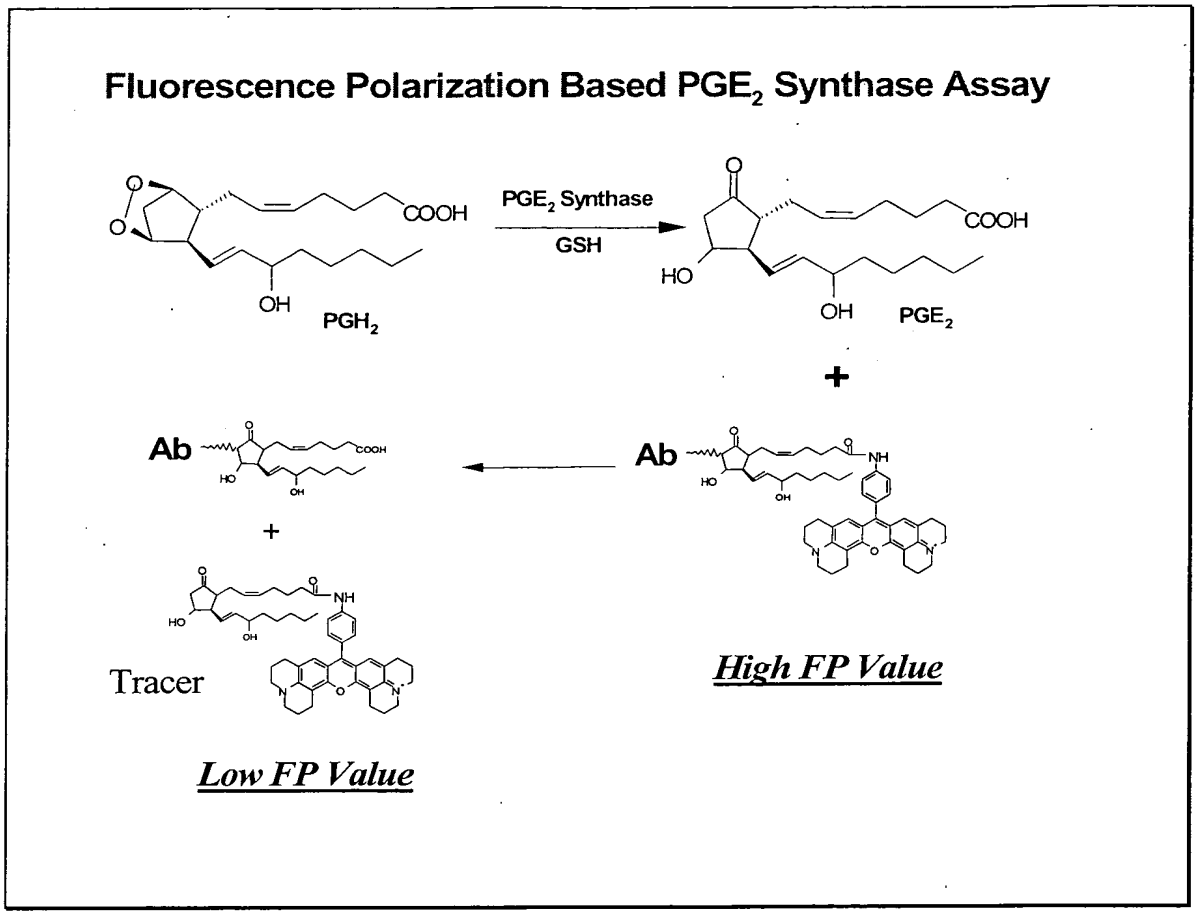

Fluorescence Polarization Based PGE2 Synthase Assay
COOH
OH
PGH2
PGE2 Synthase
GSH
HO
COOH
OH
PGE2
+
Ab
COOH
HO
OH
+
NH
HO
OH
Tracer
Ab
NH
HO
OH
High FP Value
Low FP Value

FIGURE 3

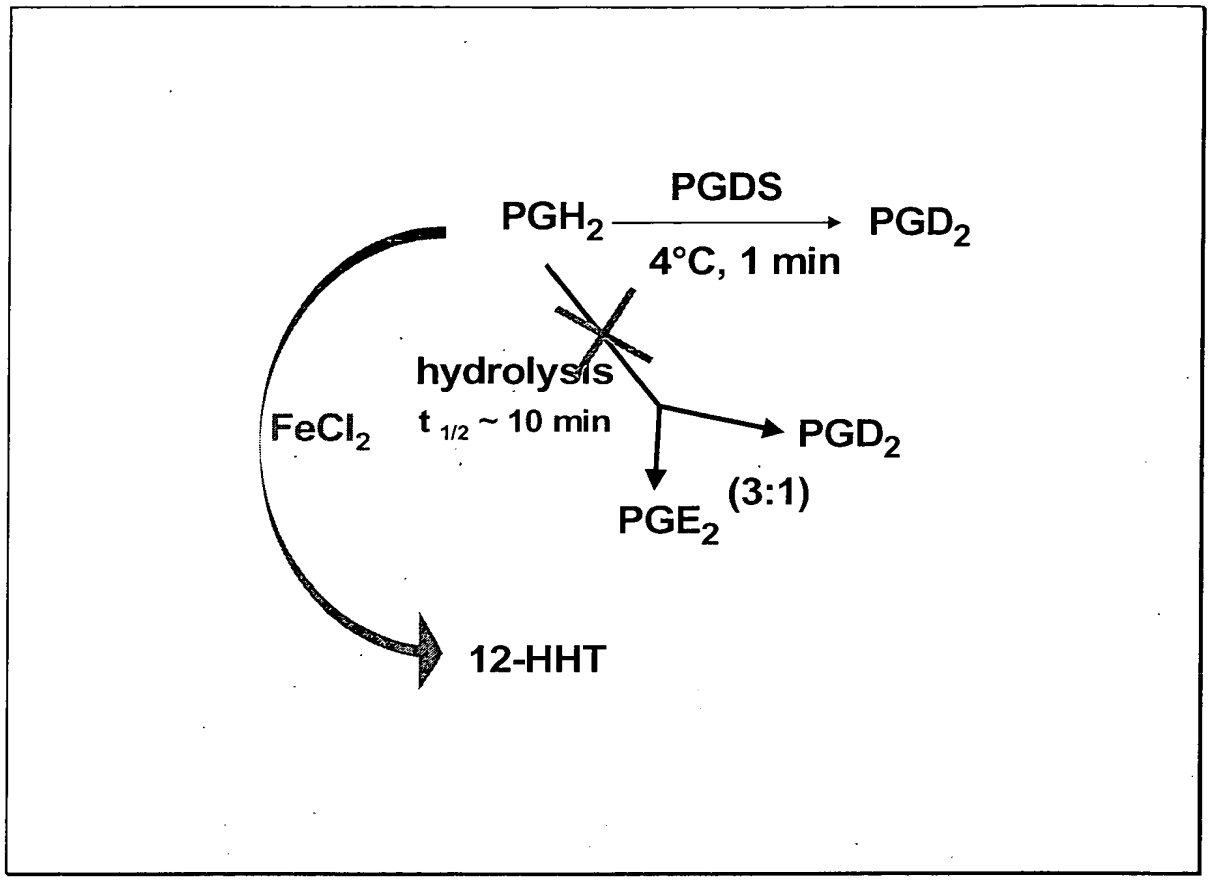

PGH2
PGDS
PGD2
4°C, 1 min
hydrolysis
t 1/2 ~ 10 min
FeCl2
PGD2
PGE2
(3:1)
12-HHT

FIGURE 4

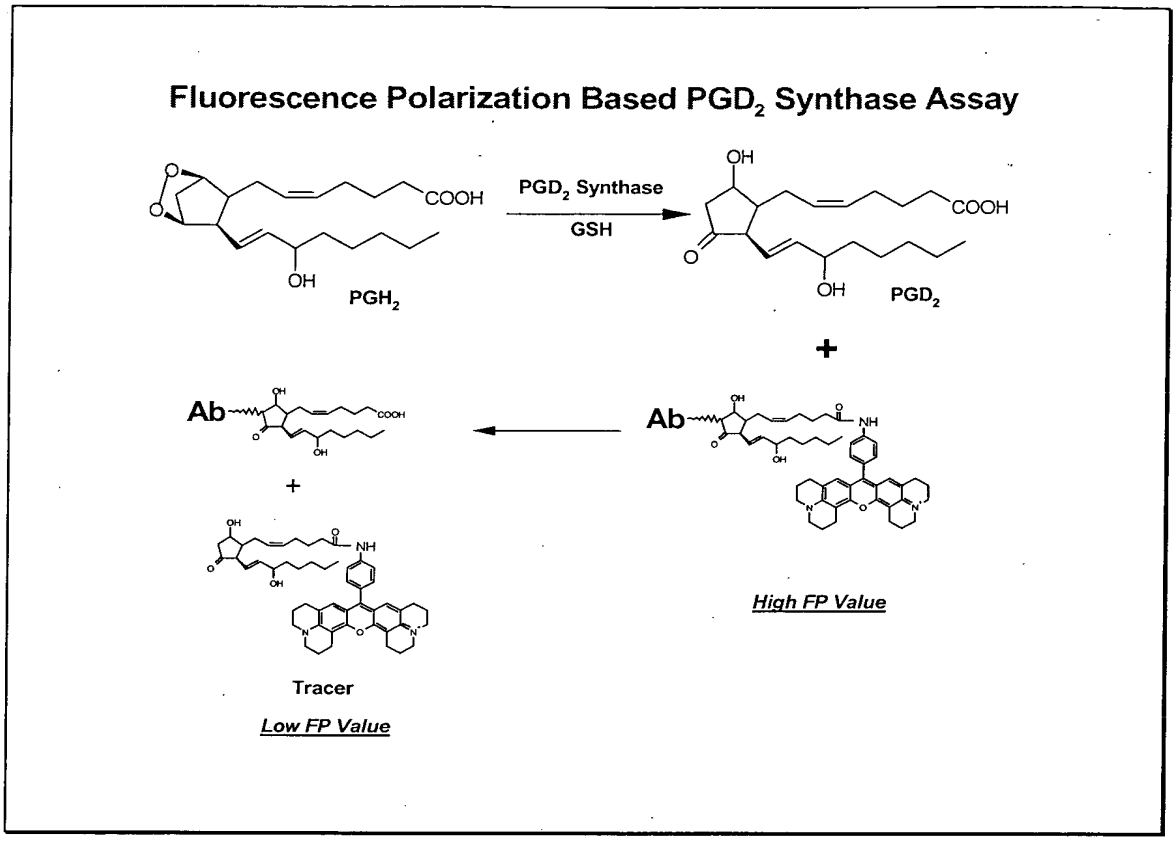

Fluorescence Polarization Based PGD2 Synthase Assay
COOH
OH
PGH2
PGD2 Synthase
GSH
OH
COOH
O
OH
PGD2
+
Ab
COOH
OH
O
+
NH
OH
O
Tracer
Low FP Value
Ab
OH
NH
O
N
O
N
High FP Value

FIGURE 5

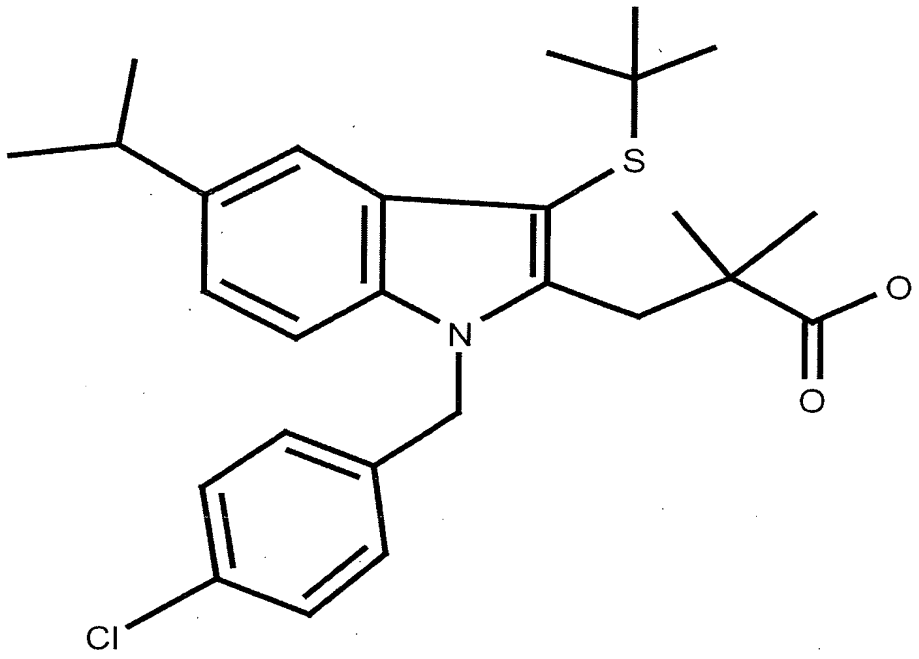


FIGURE 6

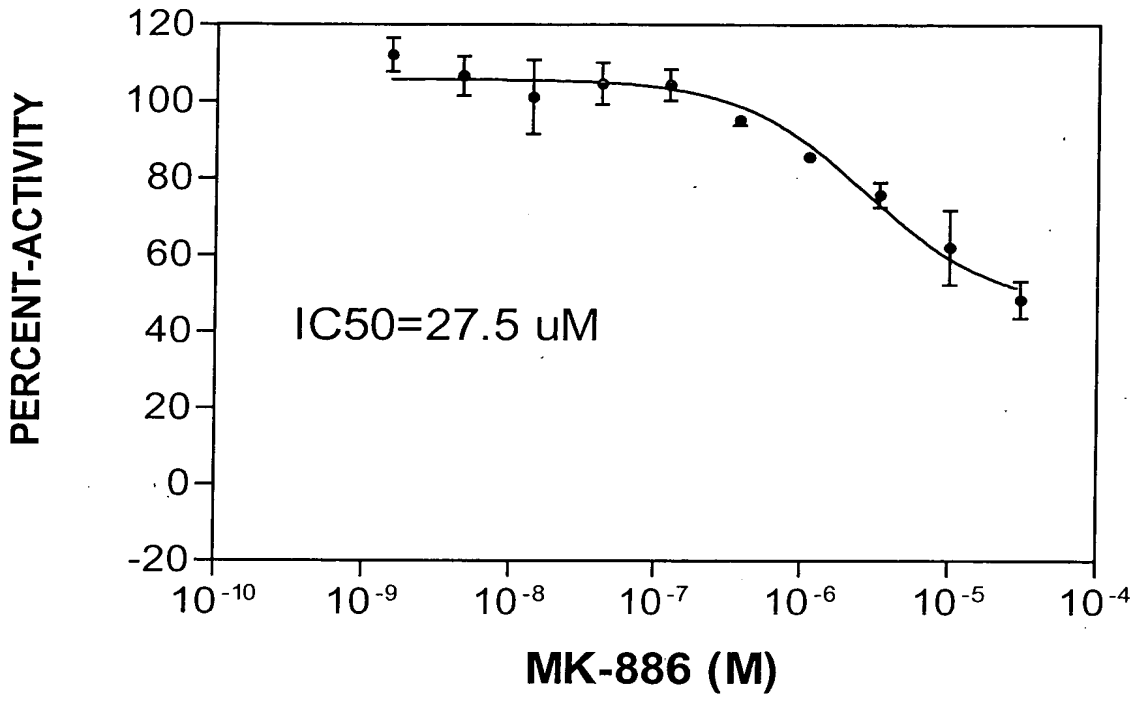

PERCENT-ACTIVITY
120
100
80
60
40
20
0
-20
IC50=27.5 uM
10-10
10-9
10-8
10-7
10-6
10-5
10-4
MK-886 (M)

FIGURE 7

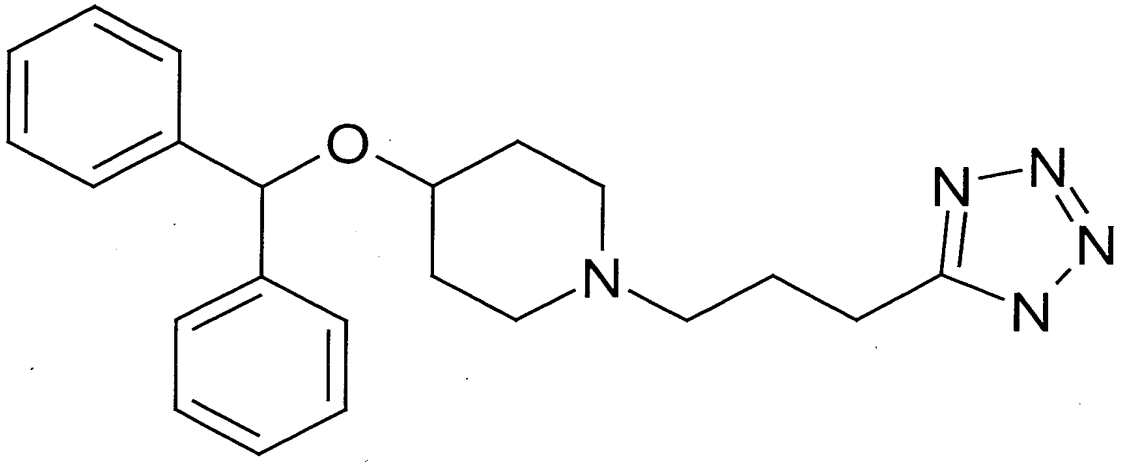


FIGURE 8